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Recombinant non-structural 1 (NS1) protein of dengue-2 virus interacts with human STAT3β protein

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Abstract

A combination of yeast two-hybrid library screening, co-immunoprecipitation and immunofluorescence microscopy demonstrated that dengue-2 virus non-structural 1 (NS1) protein can interact with an N-terminally truncated form of human STAT3β (ΔN40-STAT3β) protein. The NS1 protein interacted with the activated STAT3β protein in vesicle-like structures in the cell cytoplasm. In addition, transfection of dendritic cells with plasmid expressing NS1 protein also resulted in significant induction of tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6). Since the STAT3β protein is an acute-phase response factor, its interaction with NS1 protein may influence the pathological changes observed in dengue fever, dengue hemorrhagic fever and dengue shock syndrome.

Keywords: Dengue virus; Flaviviruses; Non-structural 1 protein; Human STAT3β protein; Protein–protein interaction; Yeast 2-hybrid

1. Introduction

Dengue viruses are members of the family *Flaviviridae* consisting of four serotypes (Kuno et al., 1998). Infections caused by one or more of the four serotypes of dengue viruses can result in dengue fever, dengue hemorrhagic fever and/or dengue shock syndrome. More than half the world's population inhabit in tropical and subtropical areas are at risk of infection, thus making dengue the most important human viral disease transmitted by arthropod vectors (Rigau-Perez et al., 1998).

Data from epidemiological studies of children suffering from dengue hemorrhagic fever indicate that hemorrhagic and hypotensive complications are more common in heterotypic secondary dengue infections than primary infections (McBride and Bielefeldt-Ohmann, 2000; Rothman and Ennis, 1999). In vitro, cells of the macrophage lineage are more readily infected with dengue viruses in the presence of sub-neutralizing levels of cross-reactive but non-neutralizing antibodies, collectively referred to as enhancing antibodies (Morens, 1994). These data led to the theory of antibody-dependent enhancement that proposes the binding of antibody-virus complexes to the $F_{C\gamma}$ receptor of monocytes and macrophages at sub-neutralizing antibody levels gives rise to increased cell surface binding, internalization and productive infection of these cells (Morens, 1994).

Being antigen-presenting cells, macrophages present viral antigens via major histocompatibility (MHC) complex class I and II molecules to $CD4^+$ and $CD8^+$ T cells. Infection leads to their activation and production of cytokines, ultimately giving rise to immunopathology. Pro-inflammatory cytokines such as interferon-γ, interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) produced by macrophages, lymphocytes and endothelial cells contribute to and aggravate this cascade of inflammatory events (McBride and Bielefeldt-Ohmann, 2000; Rothman and Ennis, 1999).

It should be noted, however, that antibody-dependent enhancement cannot fully account for the pathogenesis of

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dengue hemorrhagic fever and dengue shock syndrome. Molecular mimicry of viral proteins, notably the envelope (E) and non-structural 1 (NS1) proteins (McBride and Bielefeldt-Ohmann, 2000; Chang et al., 2002) as well as viral virulence theories (Holmes and Burch, 2000) have been proposed to contribute to the immunopathogenesis of dengue hemorrhagic fever.

Flavivirus NS1 is a 40–50 kDa glycoprotein that exists intracellularly within the perinuclear region as cytoplasmic foci, or as cell surface associated or extracellular non-virion forms (Chambers et al., 1990). A detergent stable dimer is the predominant form of both cell-associated and secreted NS1 protein although a hexameric form of the secreted dengue virus type 1 NS1 protein was recently reported (Flamand et al., 1999).

Although the functions of NS1 protein are not fully elucidated, several lines of evidence suggest that NS1 protein is involved in viral RNA replication (Chu and Westaway, 1992; Lindenbach and Rice, 1997; Mackenzie et al., 1996; Muylaert et al., 1997; Westaway et al., 1997). The NS1 protein is by far the most immunogenic of the non-structural proteins (Cardosa, 1998) and flavivirus infections elicit antibodies to NS1 protein with complement-fixing activity. Thus, the secreted form of NS1 protein called the soluble complement-fixing (SCF) antigen (Chambers et al., 1990) is detectable in circulating blood during dengue virus infection (Rice,1996).

Elevated levels of serum NS1 protein correlating with high viremia have been reported in dengue hemorrhagic fever/dengue shock syndrome patients (Libraty et al., 2002; Young et al., 2000). Besides serving as a marker of disease severity, elevated levels of NS1 protein may constitute an important factor in the pathogenesis of dengue hemorrhagic fever/dengue shock syndrome. In this study, we identified a human cellular protein (STAT3β) that interacts with dengue-2 virus NS1 protein, and propose that this interaction may play a role in the immunopathogenesis of severe dengue virus infections.

2. Methods

2.1. Cells and viruses

Dengue virus type 2 (New Guinea C strain) was propagated in the C6/36 mosquito cell line in L15 medium (Gibco BRL) containing 2% fetal calf serum. Baby hamster kidney (BHK-21 clone 13) cells were cultured in RPMI 1640 medium containing 10% fetal calf serum.

2.2. Construction of expression plasmids

Viral RNA was extracted from dengue virus-infected cell culture supernatant using LS reagent (Invitrogen), and converted to cDNA using primer D2RN and Superscript II reverse transcriptase (Invitrogen). The list of primers used throughout this study will be provided upon request.

To construct the bait plasmid used for yeast two-hybrid screening, the plasmid vector pGBKT7 (Clontech) encoding the GAL4 DNA-binding domain (BD) was employed. Dengue NS1 cDNA was amplified by high fidelity polymerase chain reaction (PCR) using Advantage 2 polymerase mix (Clontech) with primers Y1F and Y1R engineered to contain *Nde*I and *Bam*HI restriction sites, respectively. The amplified products were then digested with the appropriate restriction enzymes and inserted in-frame with the GAL4 BD of the pGBKT7 vector.

To generate the mammalian expression plasmid producing V5-tagged dengue NS1 fusion protein (VNS1), dengue NS1 cDNA was first amplified using primers 1FK and N1R. The NS1 insert was then ligated into a pcDNA3.1d plasmid (Invitrogen) containing a C-terminal V5 epitope tag via topoisomerase-mediated cloning according to the manufacturer's recommendations. The plasmid expressing dengue NS1 protein (sVNS1) containing the endogenous NS1 signal peptide sequence (sNS1) was obtained via PCR amplification of the VNS1 plasmid with primers sN1F and sN1R using *pfu* polymerase. To generate the expression plasmid for myc-tagged human ΔN40-STAT3β fusion protein (MX10), the fragment designated ΔN40-STAT3β corresponding to 94% of the opening reading frame (ORF) encoding amino acids 41–722 of the published sequence (GenBank accession number U30709) was cloned. The activation domain vector primers FAD and RADO were employed to amplify the ΔN40-STAT3β fragment from an interacting plasmid of the yeast two-hybrid library identified during yeast mating experiments. The PCR product was ligated into pCR-XL-TOPO vector (Invitrogen). The ΔN40-STAT3β-encoding insert was released using *Sfi*I and *Xho*I enzymes and reinserted into the mammalian expression vector pCMV-myc containing a myc tag (Clontech). The expression of NS1 and ΔN40-STAT3β proteins was under the control of the cytomegalovirus immediate-early promoter.

2.3. Screening of a yeast two-hybrid library

Yeast two-hybrid library screening was performed using the Matchmaker GAL4 Two-Hybrid System 3 (Clontech). AH109 yeast cells were transformed with the NS1 bait construct using the lithium acetate method. Auto-activation of the HIS3 reporter gene was detected by growth in synthetic dropout agar lacking amino acids Trp and His. Expression of the NS1-BD fusion protein was also analyzed by Western blotting.

Yeast mating assays were performed using the Matchmaker yeast pre-transformed bone marrow cDNA library (Clontech) as recommended by the manufacturer. The entire mating culture was plated onto synthetic dropout agar lacking Leu, Trp, His with or without adenine. A total of 3×10^6 clones were screened against the dengue virus NS1 bait. Colonies growing on the selection plates were then re-streaked on fresh plates. In order to eliminate false-positives, colonies were assayed for *Lac*Z reporter gene activity on